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New breast cancer cell strains and cell lines were developed from many breast cancer tissues and from uninvolved normal tissues. These cell strains/lines were added to a new cell repository for breast cancer cells and genomic DNA. Primary cultures were initiated using cell suspensions isolated from the tissues and using cellular outgrowth from explanted tissues. Various cell isolation methods and a variety of culture media and hormone combinations were tested to avoid senescence in vitro. Thirty cultures passed through the second subculture and ten of these could be expanded to a sufficient number for cryopreservation. Of these ten, six may be classified as cell strains and the other four as cell lines since they were continuously cultured for over ten subcultures. The cell lines have been partially characterized and all possess H-ras and p53 genes. Additional cells will be developed and characterized during the next grant period. Availability of these new cell lines to the research community will facilitate discovery of new therapeutic agents for breast cancer.

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Thomas Lee 9/28/95

PI - Signature Date

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INTRODUCTION

Nature of the Problem

This grant was awarded to BRFF (i) to develop a number of new human breast cell lines from cancer patients of divergent ethnicity and different stages of the diseases, and (ii) to operate a new full service repository for such cells and genomic DNA in order to make them available to the research community to do further breast cancer research. The nature of the work to achieve the two aspects supported by this grant is quite different; establishment of cell lines requires considerable research and development (R&D) work while the implementation of the cell repository requires appropriate managerial and administrative effort.

Background of Previous Work

Availability of well-characterized human mammary cells to the research community will definitely be a catalyst for stimulation of such studies using modern cellular and molecular biological concepts and other emerging technologies. It has been fairly difficult to establish human breast cancer cell lines as evidenced by the paucity of reported development of aneuploid cancer cell lines. There are a number of plausible explanations (1-4) for this difficulty, and some of the variables have been addressed in the work performed during this report period. In addition, the necessary infrastructure for the operation of the new cell repository is in place and we will open the repository to the research community during the next reporting period.

Purpose of the Present Work

The primary objective of this project is to establish, maintain and operate a repository for cell cultures and genomic DNA derived from human breast tissues. The cell collection will include both cell strains and cell lines established at BRFF using different culture media and experimental manipulations.

Methods of Approach

The cultures developed at BRFF have utilized both classical methods that favor the growth of fibroblastic and/or normal diploid epithelial cells, as well as innovative methods that selectively grow the mammary carcinoma cells. The epithelial cells are grown in special culture media supplemented with low levels of serum, as well as in serum-free media. Part of the original tissue and the cell strains are cryopreserved at the earliest possible time; cell lines from the same cell strain are expanded and again cryopreserved in larger batches. Each of the continuous (immortal) cell lines are characterized with respect to its karyology, morphology, differentiated functions and tumorigenic capacity in nude mice. To insure error-free operation of this valuable resource for cancer research, a body of comprehensive Standard Operating Procedures (SOP) governs the technical aspects of the various tasks and also the logistical aspects of tracking samples obtained from or delivered to collaborators.

BODY

Experimental Methods Used

Cell Culture Methods. Two methods were used for setting up primary cultures of the breast cancer tissue: (i) plating single-cell suspensions or (ii) attaching small pieces of the tissue as explants on specially treated growth surfaces.

For the first method, single cell suspensions were produced by progressive enzymatic dissociation of the tissue (5)or mechanical "spilling" of the cells (6). The tissue was washed free of the transport medium, minced and suspended in 10 volumes of CTC dissociation medium (contains 0.1% Collagenase (Type I Worthington Biochemicals), 0.05%Trypsin, (Type XI Sigma), and 1% Chicken serum in HEPES Buffered Saline (HBS)) in a conical flask. The minced tissue was incubated at 37°C for 30 minutes, and then isolated cells in suspension were sieved through a cell strainer (Falcon #2350) and collected by centrifugation (100 x *g* for 5 min). Dissociation of the undigested tissue was repeated two or more times and the pooled cell suspension counted and plated as the primary culture. In some cases, the cells from the tissue could be easily disaggregated by cutting the tissue into small fragments.

For the second method which utilizes explant cultures (7), the tissue was washed free of the transport medium and cut into 1-2 mm^3 fragments with crossed scalpel blades. These fragments were then transferred to culture dishes that were "scratched" with a scalpel and coated with a coating mixture (FNC) (FNC contains bovine Fibronectin, Collagen (Type 1), and bovine serum albumin in HBS). The tumor fragments were placed on the "scratched" surface approximately 1 cm apart. A small volume of growth medium was added so that the entire surface of the dish (but not the tissue fragments) was covered. These explants were incubated at 37°C in a humidified incubator with a gas phase of 5% CO_2 in air. When substantial zones of cells were formed, the explanted tissues were transferred to another dish.

For both primary and subsequent subcultures (SC), cells were plated in the specific culture medium (as dictated by the experiment) and incubated at 37°C in an atmosphere of CO_2 in air . The medium was changed 3 times a week and monolayers were subcultured when they attained confluency. Anchorage-dependent cells were detached from the plastic by a dissociation medium containing Polyvinylpyrrolidone, EGTA, and Trypsin (PET), and the tryptic activity was inhibited by addition of FBS-supplemented HBS. The cells were centrifuged and resuspended into the desired growth medium and a known inoculum size was then plated on FNC-coated plastic. The number of cells obtained at the subculture were recorded and used to prepare the Cumulative Population Doubling Levels (CPDL) shown in this report.

It was of prime importance that the cell lines be derived from the particular breast sample and not cross-contaminated by another specimen. Precautions against cross-contamination included cleaning the work surfaces with alcohol and irradiation of the Biohazard Hoods with ultraviolet light before handling each cell type, working on only one cell type at a time, and using a separate bottle of medium for each cell strain.

Cryopreservation of the breast cells. The cell suspensions were centrifuged and the pellets resuspended at a density of 4-6 x 10^6 cells per ml in ice-cold L-15 medium containing 10% FBS and 2X Gentamicin (100 mg/ml). An equal volume of ice-cold L-15 medium containing 10% FBS and 15% DMSO was added slowly (8) to the cell suspension. One ml aliquots of the cell suspension were transferred to pre-cooled cryovials, frozen slowly at a controlled rate and transferred to the vapor phase of a liquid nitrogen freezer. Samples of fresh breast tissue were also frozen and maintained at -80°C for possible future studies after establishing cell lines.

Transfection Experiments. Transfections (5) were carried out either using the newly developed cell strains or the explant cultures with pRSV-T DNA by lipofection as follows. For each dish, 5 μg DNA in 100 μl was mixed with an equal volume of diluted lipofectin or lipofectamine reagent (1:4 dilution) in a polystyrene tube and kept at room temperature for 15 min. It was then diluted to 2 ml with the culture medium. The monolayers were rinsed twice with the culture medium and then 2 ml of the DNA mixture was added drop-wise while gently swirling the dish. After 6-8 hr of incubation at 37°C, the medium was removed and replaced with regular medium and the transfected cultures were maintained using the routine conditions described above.

Morphological Studies. The cell cultures were routinely checked under a Nikon phase contrast inverted microscope and photographed. Scanned images of the photographs were captured on computer disks and printed using a laser printer .

Cytogenetic analysis. Semi-confluent cultures were sent to the Cell Culture Laboratory at Children's Hospital of Michigan for karyotypic analyses. Exponentially growing cultures were treated with 0.04 μg/ml of Colcemid for 1-2 hours, trypsinized and treated with 0.0375 M KCI for 9 minutes, and fixed in 3:1 methanol:glacial acetic acid mixture. The suspension was centrifuged and washed a couple of times with fixative and finally dropped on cold wet slides as previously reported (9). Slides were air dried and stained with 4% Giemsa solution and used for the determination of ploidy distribution, chromosome counts and constitutional aberrations. For trypsin Giemsa banding (GTG), karyotypes were prepared by a modified procedure of Seabright (10). The slides were aged at 60°C on a slide warmer for 16-20 hours, immersed in 0.025% trypsin for 1-2 seconds, stained with 4% Giemsa solution for 11 minutes, washed in buffer, dried and mounted in Permount. Well banded metaphases were photographed at X800 magnification using technical Pan film 2415 (Kodak) and printed on Rapidoprint FP 1-2 (Agfa-Geavert) or were karyotyped using the AKSII image analysis system. A minimum of 5 karyotypes were prepared from these prints and arranged according to standard karyotype (11).

Genomic DNA Isolation and PCR Amplification. Genomic DNA was isolated from the cell pellets of different cell lines following an established protocol which utilizes proteinase K digestion of the cells, phenol-chloroform extraction, and ethanol precipitation (12). The quantity and purity of the genomic DNA was then checked using a Pharmacia GeneQuant DNA calculator.

PCR amplification of the genomic DNA samples was performed in order to characterize cell

lines with respect to the presence or absence of specific proto-oncogenes and tumor suppressor genes. One μg of the genomic DNA was used in PCR reactions. PCR amplification of codons 12 and 61 of the *H-ras* oncogene was performed using Clontech's amplimer sets and the PCR protocol followed Clontech's suggested procedure. PCR amplification of the p53 tumor suppressor gene was also performed using Clontech's amplimer sets and suggested protocol. Specifically, exon 8 of the p53 gene was amplified.

Description of the Various Media Tested

During the course of this reporting period, we used different culture media based on the previous studies reported by other investigators (13,14) and by our laboratory (15-17). Different "basal media" (13,18,19) were supplemented with a number of growth factors to produce Serum-free media (7,15,20). Some of these Test Media were supplemented with added FBS at different concentrations. To minimize the possible confusion and to avoid repetition, the different media used in this study are identified below:

1. Growth Factors & Hormones	*2. Amino Acids, Vitamins, HEPES, Trace Elements, etc*	*Combination of 1 & 2*

EGF	BPE	TF	EA	PEA	HC	Insulin	Prostaglandin	Prolactin	Beta-estradiol	DHT	Basal Medium Used to make the "TEST MEDIA" in the next Column	Codes for the **"TEST MEDIA"** used in this Study
-	-	-	-	-	-	+	-	-	-	-	EMEM+NEAA+Pyruvic Acid	**EMEM**
+	+	-	+	+	+	+	-	-	-	-	MCDB (modified)	**EPM-2**
+	+	+	+	+	+	+	-	-	-	-	PFMR-4 (modified)	**BM-0**
+	+	+	+	+	+	+	-	-	-	+	PFMR-4 (modified)	**HPC-1**
+	+	+	+	+	+	+	+	+	-	-	MCDB 170	**BM**
+	+	+	+	+	+	+	-	+	+	-	PFMR-4 (modified)	**BM-3**
+	+	+	+	+	+	+	-	+	+	+	PFMR-4 (modified)	**BM-2**
+	+	+	+	+	+	+	+	+	+	-	MCDB 170	**BM-1**

Description of the Human Breast Tissue Used

During this reporting period, we have received a total of 38 samples mainly from Ohio State University. These included 24 cancer tissues of which 12 were metastatic to other organs. Thirteen uninvolved tissues from cancer patients were also obtained. In some cases, uninvolved and cancer tissue from the same patient was obtained. These and other details are listed in Table 1 in the Appendix.

If sufficient amounts of tissue were available, a small portion was frozen for comparing the DNA profile after a cell line was established. If relatively large amounts of tissue were available, a portion was cut into small pieces and cryopreserved in a modified L15 freezing medium. These cryopreserved tissues, and those received as cryopreserved samples from collaborators, are stored in the repository and an inventory is shown as Table 4 in the Appendix.

Results Obtained

Cell culture experiments were initiated in a chronological manner as the fresh tissues were received. A total of 56 primary cultures were prepared of which 18 did not go through SC1. However, three of these cultures were only initiated very recently. Although cells grew well initially, they showed terminal differentiation and could not be easily isolated from primary culture dishes. Various cell isolation methods and culture media have been tested to avoid senscence *in vitro*. About 30 cultures passed through the second subculture but only 10 of these could be expanded to a sufficient number for cyropreservation. Of these 10, 4 were cultured over subculture 10 and may be classified as cell lines. The other 6 may be classified as cell strains. These cell strains will be reconstituted and transfected with transfecting genes during the next reporting period. The entire inventory of the cryopreserved BRF cell strains/lines are given in Table 3 in the Appendix.

Some of the cell lines have been partially characterized with respect to their chromosomal profile, the retention of protooncogenes *(ras)* and tumor suppressor gene (p53). The Cumulative Population Doubling time of these cell lines has been documented and is shown in this report.

Tissue Samples and Cell Strains/Lines

The lineage and early history of the cell strains and cell lines are given in Table 2 in the Appendix. A synopsis of tissue samples (identified by BRFF number) and cell strains/lines (identified by BRF number) is given below. When isolated cell suspensions or explant culture were transfected with pRSVT, these transfected cell cultures are identified with a capital T after the BRF cell number. Any cell line established from different experimental conditions is identified by using A,B,C, *etc.* Please note that SC denotes subculture or passage number.

BRF-29. 3.9 gms of normal uninvolved breast tissue (mostly breast skin) was used (BRFF29) for preliminary experiments to study a number of cell culture variables. Since this tissue was from breast skin, EPM-2, a serum-free medium known to yield cell lines of epidermal origin (15) was used in this study. However, EPM-2 was supplemented with 1% Fetal Bovine Serum (FBS) with a view

to enrich the aneuploid cancer cells (4) that might be present in the uninvolved normal tissue from this cancer patient.

A portion of BRFF29 tissue was used to isolate cells by enzymatic digestion with *1% Collagenase in HEPES buffered saline* at *37°C* for overnight. This yielded the cell line BRF-29. This culture was maintained in EPM-2 with 10% FBS for 14 subcultures and cryopreserved at various intervals. BRF-29 will be useful as a basal layer to grow other epithelial breast cell lines. Another portion of BRFF29 tissue was treated with the same enzyme at *4°C* overnight but did not yield cell suspensions. The next variable was the use of PTC (a mixture of polyethylene glycol, trypsin and Collagenase) for cell isolation and the use of explant to produce outgrowth. The cells isolated with PTC and transfected with pRSV-T did grow well for 54 days (SC3) and these cells were cryopreserved for future use.

Another portion of the tissue was used to make explant cultures on FNC-coated petri dishes. The explant cultures BRF-29TD and BRF-29TF produced outgrowths consisting of more than one cell population. While fibroblastic cell lines BRF-29TA,B,C grew well, the epithelial cells isolated from the explant outgrowths did exhibit terminal differentiation.

BRF-30. 2.1 gms of breast skin (BRFF30) was used to repeat the previous experiment with BRF-29 with minor modifications. PTC was injected into the tissue and incubated overnight at room temperature. Cells isolated by this method were not viable and did not yield any cell cultures. Portions of this tissue were cut and cryopreserved for possible use later in this study.

BRF-31. 0.5 gms of breast tissue from a *male* and used as explant culture in EPM2 supplemented with 1% FBS (as in BRF-29) but did not yield any outgrowths. The tissue was mostly fat.

BRF-32. A very small piece of breast tissue and cells isolated using 0.25% trypsin at 4°C overnight, but did not separate the epidermis from the dermis. Some cells "spilled" into the medium after they were cut into small pieces. These cells also did not grow well (may be because of the overnight treatment with trypsin). Another variable tested was the use of BM or EPM-2 without any added serum.

The tissues used for the next three cell preparation (BRFF34, 35 and 36) were obtained from the same patient.

BRF- 34. 0.7 gms of a Lymph Node Metastatsis was received. Explant cultures were set up in BM medium. No outgrowth was observed for over one month.

BRF-35. 1.4 gms of uninvolved tissue from the same patient as in BRF34 was used as explant cultures, as well as, for cell isolation with CTC. The CTC-dissociated cells did not grow in cultures. Tissues were incubated in "trypsinization flasks" for two 30 min. periods. The cell recovery was very poor which may be a reflection of the short period of incubation.

The explant cultures in BM produced cellular outgrowths. These explant cultures were

transfected with pRSV-T. The growth continued and the cells were subcultured using PET. However, the cells in one group (BRF-35TA) were purely fibroblastic, hence they were discarded. BRF35-TB cells were discarded due to fungal contamination.

BRF-36. A small piece of skin from BRFF36 was used for cell isolation with overnight incubation with CTC at room temperature. The cells were grown in EPM-2 + 10% FBS. The fibroblastic cells from this culture were cryopreserved and the experiment discontinued.

BRF-44. 0.3 gms of primary breast cancer (BRFF 44) was set up as explants in BM-1 (MCDB supplemented with β-estradiol) and in the control BM. There were only very few cells in the outgrowths. FBS was added intermittently with a view to enhance cell growth but the results were not significant.

BRF-46. 0.3 gms of an invasive breast cancer (BRFF 46) from a Black patient was set up as explant cultures in 3 types of culture media (BM-1, HPC-1, and 1:1 mixture of BM-1:HPC-1). Good outgrowths were seen from some of the explants maintained in HPC-1, the other two conditions showed significantly less outgrowth. We have then subjected the "non-growing" explants to enzymatic digestion with Collagenase and Dispase (37° shaking overnight) and used the isolated cells as cell cultures. The effects of various culture media (BM-0, BM2, BM2 + 5% FBS) were then studied using this culture after it had been transfected (BRF46TA). This cell line ultimately became dendritic and had to be discarded.

The explant cultures maintained in HPC-1 were also transfected (BRF-46TB) and the morphology of the progeny were purely epithelial. These cultures were used to study the effect of a new medium BM-2 (containing β-estradiol, Prolactin and Dihydrotestosterone). This strain also had to be discarded because of the difficulty in cell detachment required for subculturing the cell outgrowths.

BRF-47. 0.5 gms of primary breast cancer were used to set up explants and spill cultures. The effect of BM-1 and BM-2 were tested using these cells. BRF-47A in BM-2 showed a better growth and was used to obtain the transfected line BRF-47TI at subculture 1. After the second subculturing BRF-47A did not grow. On the other hand, BRF-47TI was passaged until SC6 and was cryopreserved. However, cultures from two T-25 flasks have not been subcultured for over 2 months. The number of cells has not increased at all. Explants grown in BM2 did produce good outgrowth but did not survive the subculture procedure. Even the transfected cells did not form a cell line.

BRF-49. 14.5 gms of metastatic breast cancer (BRFF 49) into the right lobe of liver was received and used for cell cultures and explant cultures. Since the tissue was primarily composed of liver, we have used the isolation mixture (Collagenase/Dispase) found to be good for liver cell isolation for cell isolation in the trypsinization flask. Cells were extracted after every 30 minutes. Cells were pooled and plated in HPC-1, BM-1 and BM-2. Cells in HPC-1 grew well for 4 passages. The cells were cryopreserved at SC1. BRF49-TD (obtained from SC2 of the BRF-49A) was growing well in BM-2. It had to be stopped due to fungal contamination.

BRF-50. 0.5 gms of metastatic breast cancer (BRFF 50) to the ovary was set up as explant cultures

but could not be grown.

The tissues used for the next three cell preparations (BRFF51,52,53) were obtained from the same patient. Tissue 51 was mainly fat. Explants were set up from all, but none gave any cellular outgrowth at all. BRFF 64 tissue was contaminated with yeast and had to be discarded.

BRF-65. 0.2 gms of Lymph Node Metastasis (BRFF 65) was set up as explants only. The effect of BM-0 (with no hormone), with male hormone DHT only (HPC-1) with female hormones only (BM-3) and with all three hormones (BM-2) were tested using these cultures. Good outgrowths were present in all groups but it was difficult to rank order their effects. At the first subculture, cells grown in BM-2 gave the highest cell number. BRF-65A was grown in BM-2. Similarly, another transfected cell, BRF-65TF, also grown in BM-2 gave a high cell count at SC1. Unfortunately all the 65 series had to be abandoned due to a fungal infection.

BRF-69. 0.4 gms of a Lymph Node Metastasis of breast cancer (BRFF 69) was set up as explant cultures in BM-2 only (because of the previous good results). This tissue along with the cellular outgrowth was transfected (BRF-69TC) and cultured continually for subculture 12. The cells were cryopreserved at SC2 and DNA extracted at SC5. Although this culture has exhibited certain signs of senescence at SC10, it is still being cultured at present. Much additional information such as the chromosomal profile, and CPDL, *etc.* are known. Recently, the cryopreserved cell line has been reconstituted for further characterization.

BRF-71. 0.9 gms of Lymph Node Metastasis (BRFF 71) was set up as spill culture (BRF-71A) and explants in BM-2. The spill cultures did not grow well. Although SC1 was reached, this culture finally did not grow any further. However, explants with cellular outgrowth transfected with pRSV-T grew continuously. The two cell lines (BRF-71TB and TC) were practically the same except they were derived from two separate transfections. BRF-71TC was enriched for epithelial cells by selective subculture and studied in detail. Although this culture has exhibited certain signs of senescence at SC10, it is still being cultured at present. Much additional information such as the chromosomal profile, and CPDL, *etc.* are known. Recently, the cryopreserved cell line has been reconstituted for further characterization.

BRF-72 to 77 were received from India (BRFF 72-77) as cryopreserved pieces of various breast cancer tissues.

BRF-72 was reconstituted and explants were set up in BM-2. This tissue did not produce any cellular outgrowths. We have not yet processed BRFF73 - 77.

BRFF79 and 80 were obtained from the same patient. 0.3 gms of uninvolved breast skin was set up as explants in EPM-2 and transfected as explants with pRSV-T. The cells are still growing well.

BRFF80 was also set up as explants in BM-2 and no cellular outgrowths were seen. This tissue was very hard to cut into explants.

BRFF81. 0.2 gms of primary breast cancer was set up as explant cultures in BM-0 and BM-3. All explants showed cellular aggregates within the loose connective tissue. Only in the BM-0 dish, was

there any cellular outgrowth.

BRFF82. A large sample of normal breast tissue from a young black woman was obtained for comparative studies. The tissue was mostly fat. After defatting, the tissue was used to set up explant cultures and cell cultures derived from primary cells isolated by CTC treatment for 6.5 hours at 37°C in trypsinizing flasks. The explant cultures were found to be mostly fat and were discarded. BRF-82C has grown slowly to SC2 in BM-3. The transfected equivalent, BRF-82TB is now in SC4 and growing well.

BRFF84. 0.4 gms of primary breast cancer was used to set up explants in BM-2. The cellular outgrowth is growing at a slow rate.

The tissues used for the next three cell preparation (BRFF 86, 87) were obtained from the same patient.

BRF-86. 0.5 gams of primary breast cancer was used to set up spill cultures and explants. Both are now at SC2.

BRF-87. 10.7 gms of uninvolved breast tissue was used to set up cell cultures using primary cells isolated by CTC digestion for 6 hours at 37°C in a trypsinization flask. The culture is in BM-2 and cells were cryopreserved at SC1. The culture is still being continued.

BRF-88. 5.4 gms of uninvolved breast tissue from a cancer patient were set up as cell cultures after CTC digestion. Both control (BRF-88) and transfected cell (BRF-88T) in BM-0 have passed SC2. Explant is still in progress.

BRF-89. This tissue was cryopreserved at Ohio (BRFF 89) and small pieces from the reconstituted sample were washed free of freezing medium and set up directly as explant cultures in BM-0 and BM-2. Cellular outgrowths were seen after 2 weeks in cultures. Explants are still in progress.

BRF-96. 0.7 gms of primary breast cancer (BRFF 96) were set up as spill cultures and explant cultures in BM-2 and BM-0. BRF-96A, B and BT are being grown.

BRF-98. 3 gms of uninvolved breast tissue (BRFF 98) from a cancer patient were used to set up explant cultures in BM-0 and BM-3. There is already outgrowth after 11 days. Explants are still in progress.

BRF-99. 2.3 gms of uninvolved breast tissue (BRFF 99) from a cancer patient were used to set up explant cultures in BM-0 and BM-3. There is already outgrowth after 11 days. Explants are still in progress.

BRF-100. 10.8 gms of uninvolved breast tissue (BRFF 100)from a cancer patient were used to set up explant cultures in BM-0 and BM-3. There is already outgrowth after 11 days. Explants are still in progress.

Chromosomal Studies

Chromosomal studies of two cell lines (BRF-69 TC and BRF-71 TC) were performed.

BRF 69 TC (SC4). This cell line is aneuploid human female (XX), with most chromosome counts in the diploid range. The cells contain 1 or 2 copies of N3, N5, N7, N14, N15 and N16, and 2 copies of Nl, N2, N4, N6, N8-N13 and N17-N22. Deletions were observed in one of the #3 chromosomes but, the deleted segment is variable. Cells other than those of cell line BRF 69 TC (SC4) are not detected in the culture. Exact chromosome counts/30 metaphases is given in Chart 1A. Nine Giemsa banded karyotypes were prepared from metaphases with 45 (six), 46 (two) and 48 chromosomes, respectively. Photograph of one karyotype is given in Chart 1B. Deletions were observed in one of the #3 chromosomes but, the deleted segment was variable. This deletion was observed in six karyotypes and is marked as M in Chart 1B.

BRF71 TC (SC5). This cell line is aneuploid human female (XtX), with most chromosome counts in the diploid range. The cells contain1copy of N13, 1 or 2 copies of N17, and 2 copies of Nl-N12, N14-NI6 and N18-N22. There is one marker chromosome which contain various portions of the missing normal X chromosome. Cells other than those of cell line BRF 71 TC (SC5) are not detected in the culture. Exact chromosome counts/30 metaphases is given in Chart 2A. Nine Giemsa banded karyotypes were prepared from metaphases with 43 (two), 44 (two) and 45 (five) chromosomes, respectively. Photograph of one karyotype is given in Chart 2B. One marker chromosome was found showing five variants.

DNA analysis

Genomic DNA has been isolated from three different cell lines and this DNA is now stored in the DNA bank . These genomic DNA samples were then subjected to PCR amplification in order to elucidate the presence or absence of a proto-oncogene (PO), *H-ras* gene, and a tumor suppressor gene (SG), p53 gene. We observed the presence of specific *H-ras* PCR products and a specific p53 PCR product in genomic DNA samples from BRF69TC, BRF71TB, and BRF71TC. According to our grant, we proposed to classify cell lines into four categories: PO+SG+, PO-SG+, PO+SG-,PO-SG- where + indicates the presence of the gene and - indicates the absence of the gene. BRF69TC, BRF71TB, and BRF71TC are in the PO+SG+ category.

Growth rate of the cell lines

Current Cumulative Population Doubling Levels (CPDL) of the breast cancer cell lines are given in Chart 3. All the cell lines have been in culture for more than 100 days. At 100 days, the predominantly diploid cell line, BRF-69TC, had undergone about 30 CPDL while the other two had undergone over 50 CPDL. The growth rates of the BRF-71s have gone down and many detached cells are seen in the culture medium.

Discussion in Relation to the Goals of Research

A number of problems were encountered during this study and various approaches have been implemented. The cells grow very well when plated on FNC-coated dishes. However, they attach so strongly that it is difficult to isolate them for subculture. We have varied the concentrations of each component in the FNC coating mixture. In these new coating mixtures, it was still difficult to detach the cells. We propose to grow primary cells in perfusion cultures with a view to make three dimensional cultures that may dissociate better. We also propose to obtain cryopreserved cancer tissues from more sources which will allow us to perform additional R&D experiments aimed at a mass production of the cells.

CONCLUSIONS

Summary of the implication of the completed research

The R&D effort for the establishment of new cell strains and cell lines progressed at the rate envisioned in the grant application. At the present time, we have developed 6 cell strains and 4 cell lines. Two of these cell lines have been partially characterized. Moreover, new cell cultures are being developed and experiments for the characterization of some of these cell lines are in progress.

All managerial and administrative aspects of the new BRFF repository for breast cancer cells and genomic DNA have been implemented. These include (i) the purchase of an additional liquid nitrogen freezer, (ii) setting up of a computerized inventory system for the materials and (iii) a tracking system for the receipt of tissues and cells to BRFF and dispersal of cells and DNA to breast cancer researchers. In addition, ongoing SOPs for the various functions of the repository services have been adapted for the new breast cancer cell repository.

Within the next 4-6 months we expect to provide the fully characterized cell lines to interested researchers. We also plan to announce the general availability of the new human breast cell lines during the next report period.

Future Work and Recommended Changes

Future work will proceed as originally planned. Some of the cryopreserved breast cancer cell strains will be reconstituted and transfected with mutated *ras* oncogenes. Such transfections will also be performed with the new cell cultures to be initiated during the next report period.

No changes in the original plan are required for this study.

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APPENDIX

Table 1. Background of the Breast Tissue

Tissue Information			Donor Information				
Tissue ID	Diagnosis	Tissue From	Background	Age	Sex	Race	Environment
BRFF029	Uninvolved Normal	Breast Skin	Cancer Patient	33	Female	Caucasian	Ohio
BRFF030	Uninvolved Normal	Breast Skin	Cancer Patient	69	Female	Caucasian	Ohio
BRFF031	Uninvolved Normal	Breast Skin	Cancer Patient	57	Male	Caucasian	Ohio
BRFF032	Uninvolved Normal	Breast Skin	Cancer Patient	34	Female	Caucasian	Ohio
BRFF034	Metastatic	Lymph Node	Cancer Patient	52	Female	Caucasian	Ohio
BRFF035	Uninvolved Normal	Breast	Cancer Patient	52	Female	Caucasian	Ohio
BRFF036	Uninvolved Normal	Breast Skin	Cancer Patient	52	Female	Caucasian	Ohio
BRFF044	Carcinoma	Primary Breast	Cancer Patient	74	Female	Caucasian	Ohio
BRFF046	Carcinoma	Invasive	Cancer Patient	51	Female	Black	Ohio
BRFF047	Carcinoma	Primary Breast	Cancer Patient	48	Female	Caucasian	Ohio
BRFF049	Metastatic	Liver	Cancer Patient	57	Female	Caucasian	Ohio
BRFF050	Metastatic	Ovary	Cancer Patient	69	Female	Caucasian	Ohio
BRFF051	Uninvolved Normal	Breast	Cancer Patient	72	Female	Black	Ohio
BRFF052	Carcinoma	Primary Breast	Cancer Patient	72	Female	Black	Ohio
BRFF053	Metastatic	Lymph Node	Cancer Patient	72	Female	Black	Ohio
BRFF064	Metastatic*	Ovary	Cancer Patient	66	Female	Caucasian	Ohio
BRFF065	Metastatic	Lymph Node	Cancer Patient	32	Female	Caucasian	Ohio
BRFF069	Metastatic	Lymph Node	Cancer Patient	55	Female	Caucasian	Ohio
BRFF071	Metastatic	Lymph Node	Cancer Patient	44	Female	Caucasian	Ohio
BRFF072	Metastatic**	Lymph Node	Cancer Patient	75	Female	Asian Indian	Kerala,India
BRFF073	Carcinoma**	Primary Breast	Cancer Patient	75	Female	Asian Indian	Kerala,India
BRFF074	Metastatic**	Lymph Node	Cancer Patient	42	Female	Asian Indian	Kerala,India
BRFF075	Carcinoma**	Primary Breast	Cancer Patient	42	Female	Asian Indian	Kerala,India
BRFF076	Carcinoma**	Primary Breast	Cancer Patient	65	Female	Asian Indian	Kerala,India
BRFF077	Metastatic**	Lymph Node	Cancer Patient	65	Female	Asian Indian	Kerala,India
BRFF079	Uninvolved Normal	Breast Skin	Cancer Patient	57	Female	Caucasian	Ohio
BRFF080	Cancer	Primary Breast	Cancer Patient	57	Female	Caucasian	Ohio
BRFF081	Cancer	Primary Breast	Cancer Patient	63	Female	Caucasian	Ohio
BRFF082	Normal	Breast	Reduct. Mammoplasty	27	Female	Black	Ohio
BRFF084	Cancer	Primary Breast	Cancer Patient	71	Female	Caucasian	Ohio
BRFF086	Cancer	Primary Breast	Cancer Patient	84	Female	Caucasian	Ohio
BRFF087	Uninvolved Normal	Breast	Cancer Patient	84	Female	Caucasian	Ohio
BRFF088	Uninvolved Normal	Breast	Cancer Patient	40	Female	Caucasian	Ohio
BRFF089	Metastatic**	Lymph Node	Cancer Patient	57	Female	Caucasian	Ohio
BRFF096	Cancer	Primary Breast	Cancer Patient	64	Female	Caucasian	Ohio
BRFF098	Uninvolved Normal	Breast	Cancer Patient	34	Female	Caucasian	Ohio
BRFF099	Uninvolved Normal	Breast	Cancer Patient	53	Female	Not given	Ohio
BRFF100	Uninvolved Normal	Breast	Cancer Patient	45	Female	Caucasian	Ohio

* Received Contaminated
** Received as Cryopreserved Tissues

Table 2. Early History of the Breast Cell Strains/Lines

Cell ID	Culture Started On:	Culture Started As	Interval (in days) between Start Date and Sub-Culture Dates						Cryopre-served
			SC1	SC2	SC3	SC4	SC5	SC10	
BRF29	10/05/94	Cell Cuture	12	16	23	33	62	**97**	.T.
BRF29TA	10/05/94	Cell Cuture	16	36	54				.T.
BRF29TB	10/05/94	Cell Cuture	16	36	54				.T.
BRF29TC	10/05/94	Cell Cuture	16	36	54				.T.
BRF29TD	10/05/94	Explants	36						.F.
BRF29TF	10/05/94	Explants	61	63					.F.
BRF30	10/11/94	Explants	0						.F.
BRF31	10/20/94	Explants	0						.F.
BRF32	10/25/94	Explants	0						.F.
BRF34	10/26/94	Explants	0						.F.
BRF35TA	10/26/94	Explants	22						.F.
BRF35TB	10/26/94	Explants	19						.F.
BRF36	10/26/94	Cell Cuture	22	30					.T.
BRF44	12/10/94	Explants	0						.F.
BRF46TA	01/24/95	Cell Cuture	111	118	122	128			.F.
BRF46TB	01/24/95	Explants	66						.F.
BRF47TI	01/24/95	Cell Cuture	48	56	66	77	111		.T.
BRF47A	01/24/95	Cell Culture Spill	20	48					.F.
BRF47D	01/24/95	Explants	24						.F.
BRF49A	02/03/95	Cell Cuture	14	38	48	88			.T.
BRF49TD	02/03/95	Cell Cuture	14	40	59	98			.F.
BRF50	02/07/95	Explants	0						.F.
BRF51	02/10/95	Explants	0						.F.
BRF52	02/10/95	Explants	0						.F.
BRF53	02/10/95	Explants	0						.F.
BRF65A	03/16/95	Explants	21	40	46				.F.
BRF65B	03/16/95	Explants	21	33					.F.
BRF65C	03/16/95	Explants	21	33					.F.
BRF65D	03/16/95	Explants	21	33					.F.
BRF65E	03/16/95	Explants	21	33					.F.

Table 2. Early History of the Breast Cell Strains/Lines

Cell ID	Culture Started On:	Culture Started As	Interval (in days) between Start Date and Sub-Culture Dates						Cryopre-served
			SC1	SC2	SC3	SC4	SC5	SC10	
BRF65TF	03/16/95	Explants	21	40	46				.F.
BRF69TC	04/04/95	Explants	49	64	72	80	90	**143**	.T.
BRF71A	04/20/95	Cell Culture Spill	47						.F.
BRF71TB	04/20/95	Explants	26	33	39	46	54	**88**	.T.
BRF71TC	04/20/95	Explants	26	33	46	53	57	**88**	.T.
BRF72	06/07/95	Explants	0						.F.
BRF79TA	07/21/95	Explants	24	42	46	56			.F.
BRF79TB	07/21/95	Explants	26	49					.F.
BRF80	07/21/95	Explants	0						.F.
BRF81	08/01/95	Explants	0						.F.
BRF81TA	08/01/95	Explants	0						.F.
BRF82C	08/11/95	Cell Cuture	14	25					.F.
BRF82TB	08/11/95	Cell Cuture	17	31	38	45			.F.
BRF84	08/23/95	Explants	0						.F.
BRF86TA	08/31/95	Cell Culture Spill	12	20					.F.
BRF86	08/31/95	Explants	14	25					.F.
BRF87	08/31/95	Cell Culture	11	25					.F.
BRF88	08/31/95	Cell Cuture	11	22					.F.
BRF88T	08/31/95	Cell Cuture	11	20					.F.
BRF89	08/31/95	Explants	0						.F.
BRF96A	09/13/95	Cell Culture Spill	12						.F.
BRF96B	09/13/95	Explants	12						.F.
BRF96BT	09/13/95	Explants	12						.F.
BRF98	09/15/95	Explants	0						.F.
BRF99	09/15/95	Explants	0						.F.
BRF100	09/15/95	Explants	0						.F.

Table 3. Inventory of the Human Breast Cell Strains/Lines At BRFF

BRF-ID#	SC#	Date Frozen	F	R	B	R	C	# Cells	Unit #
BRF29	01	10/28/94	12	03	01	1	A	2E6	1
BRF29	01	10/28/94	12	03	01	2	A	2E6	1
BRF29	01	10/28/94	12	03	01	3	A	2E6	1
BRF29	01	10/28/94	12	03	01	4	A	2E6	1
BRF29	01	10/28/94	12	03	01	5	A	2E6	1
								Total # of Units	5
BRF29	02	12/01/94	12	03	01	4	B	2E6	1
BRF29	02	12/01/94	12	03	01	5	I	2E6	1
BRF29	02	12/01/94	12	03	01	6	I	2E6	1
BRF29	02	12/01/94	12	03	01	7	I	2E6	1
BRF29	02	12/01/94	12	03	01	8	I	2E6	1
								Total # of Units	5
BRF29	06	12/27/94	12	03	01	6	A	2.3E6	1
								Total # of Units	1
BRF29	08	01/10/95	12	03	02	6	B	1.25E6	1
BRF29	08	01/10/95	12	03	02	7	B	1.25E6	1
								Total # of Units	2
BRF29	10	01/24/95	12	03	02	8	B	2E6	1
BRF29	10	01/24/95	12	03	02	9	B	2E6	1
BRF29	10	01/24/95	12	03	02	1	C	2E6	1
BRF29	10	01/24/95	12	03	02	2	C	2E6	1
								Total # of Units	4
BRF29	11	02/01/95	12	03	02	3	C	1E6	1
BRF29	11	02/01/95	12	03	02	4	C	1E6	1
								Total # of Units	2
BRF29TA	03	11/28/94	12	03	01	1	B	2E6	1
								Total # of Units	1

Table 3. Inventory of the Human Breast Cell Strains/Lines At BRFF

BRF-ID#	SC#	Date Frozen	F	R	B	R	C	# Cells	Unit #
BRF29TB	03	11/28/94	12	03	01	2	B	1.5E6	1
								Total # of Units	1
BRF29TC	03	11/28/94	12	03	01	3	B	2E6	1
								Total # of Units	1
BRF36	02	11/25/94	12	03	01	2	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	3	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	4	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	5	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	6	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	7	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	8	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	9	G	2.3E6	1
BRF36	02	11/25/94	12	03	01	1	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	2	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	3	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	4	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	5	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	6	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	7	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	8	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	9	H	2.3E6	1
BRF36	02	11/25/94	12	03	01	1	I	2.3E6	1
BRF36	02	11/25/94	12	03	01	2	I	2.3E6	1
BRF36	02	11/25/94	12	03	01	3	I	2.3E6	1
								Total # of Units	20
BRF47T	04	05/08/95	12	03	02	5	I	1.12E6	1
								Total # of Units	1
BRF49	02	03/01/95	12	03	02	5	E	1.4E6	1
BRF49	02	03/01/95	12	03	02	6	E	1.4E6	1

Table 3. Inventory of the Human Breast Cell Strains/Lines At BRFF

BRF-ID#	SC#	Date Frozen	F	R	B	R	C	# Cells	Unit #
								Total # of Units	2
BRF69TC	02	06/15/95	12	03	04	2	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	3	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	4	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	5	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	6	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	7	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	8	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	9	F	3E6	1
BRF69TC	02	06/15/95	12	03	04	1	G	3E6	1
BRF69TC	02	06/15/95	12	03	04	2	G	3E6	1
BRF69TC	02	06/15/95	12	03	04	3	G	3E6	1
BRF69TC	02	06/15/95	12	03	04	4	G	3E6	1
BRF69TC	02	06/15/95	12	03	04	5	G	3E6	1
BRF69TC	02	06/15/95	12	03	04	6	G	3E6	1
								Total # of Units	14
BRF71TB	03	05/30/95	12	03	03	2	I	1.3E6	1
BRF71TB	03	05/30/95	12	03	03	3	I	1.3E6	1
BRF71TB	03	06/02/95	12	03	04	1	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	2	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	3	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	4	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	5	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	6	A	2E6	1
BRF71TB	03	06/02/95	12	03	04	7	A	2E6	1
								Total # of Units	9
BRF71TB	05	06/13/95	12	03	04	4	D	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	5	D	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	6	D	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	7	D	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	8	D	2.5E6	1

Table 3. Inventory of the Human Breast Cell Strains/Lines At BRFF

BRF-ID#	SC#	Date Frozen	F	R	B	R	C	# Cells	Unit #
BRF71TB	05	06/13/95	12	03	04	9	D	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	1	E	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	2	E	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	3	E	2.5E6	1
BRF71TB	05	06/13/95	12	03	04	4	E	2.5E6	1
								Total # of Units	10
BRF71TC	04	06/12/95	12	03	04	8	B	4E6	1
BRF71TC	04	06/12/95	12	03	04	9	B	4E6	1
BRF71TC	04	06/12/95	12	03	04	1	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	2	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	3	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	4	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	5	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	6	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	7	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	8	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	9	C	4E6	1
BRF71TC	04	06/12/95	12	03	04	1	D	4E6	1
BRF71TC	04	06/12/95	12	03	04	2	D	4E6	1
BRF71TC	04	06/12/95	12	03	04	3	D	4E6	1
								Total # of Units	14
BRF71TC	10	07/24/95	12	03	05	5	F	2E6	1
BRF71TC	10	07/24/95	12	03	05	6	F	2E6	1
BRF71TC	10	07/24/95	12	03	05	7	F	2E6	1
BRF71TC	10	07/24/95	12	03	05	8	F	2E6	1
BRF71TC	10	07/24/95	12	03	05	9	F	2E6	1
BRF71TC	10	07/24/95	12	03	05	1	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	2	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	3	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	4	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	5	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	6	G	2E6	1

Table 3. Inventory of the Human Breast Cell Strains/Lines At BRFF

BRF-ID#	SC#	Date Frozen	F	R	B	R	C	# Cells	Unit #
BRF71TC	10	07/24/95	12	03	05	7	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	8	G	2E6	1
BRF71TC	10	07/24/95	12	03	05	9	G	2E6	1
								Total # of Units	14

Table 4. Inventory of Breast Tissues Stored at BRFF

Tissue ID	Date Frozen	Freeze Method	F	R	B	No. of Aliquots	Aliquots Remaining
BRFF029	10/05/94	Viable	6	6	1	10	10
BRFF030	10/11/94	Viable	6	6	1	1	1
BRFF044	12/10/94	Non viable	6	6	4	1	1
BRFF046	01/24/95	Non viable	6	6	4	1	1
BRFF047	01/24/95	Non viable	6	6	4	1	1
BRFF049	02/03/95	Non viable	6	6	4	1	1
BRFF050	02/07/95	Non viable	6	6	4	1	1
BRFF051	02/10/95	Non viable	6	6	1	2	2
BRFF052	02/10/95	Non viable	6	6	4	1	1
BRFF053	02/10/95	Non viable	6	6	4	1	1
BRFF064	03/09/95	Non viable	6	6	3	1	1
BRFF065	03/16/95	Non viable	6	6	3	1	1
BRFF069	04/04/95	Non viable	6	6	3	1	1
BRFF071	04/20/95	Non viable	6	6	3	1	1
BRFF071T	06/05/95	Non viable	6	6	3	1	1
BRFF072	06/17/95	Non viable	12	3	7	1	1
BRFF073	02/07/95	Viable	12	3	7	2	2
BRFF074	02/15/95	Viable	12	3	7	2	2
BRFF075	02/15/95	Viable	12	3	7	2	2
BRFF076	02/15/95	Viable	12	3	7	1	1
BRFF077	02/15/95	Viable	12	3	7	1	1
BRFF079	07/21/95	Non viable	6	6	1	1	1
BRFF080	07/21/95	Non viable	6	6	4	1	1
BRFF081	08/01/95	Non viable	6	6	3	1	1
BRFF084	08/23/95	Non viable	6	6	4	1	1
BRFF086	08/31/95	Non viable	6	7	7	1	1
BRFF087	08/31/95	Non viable	6	7	5	2	2
BRFF088	08/31/95	Non viable	6	7	5	1	1
BRFF096	09/13/95	Non viable	6	7	7	1	1
BRFF098	09/15/95	Non viable	6	7	7	1	1
BRFF099	09/15/95	Non viable	6	7	5	1	1
BRFF100	09/15/95	Non viable	6	7	5	1	1

LEGENDS

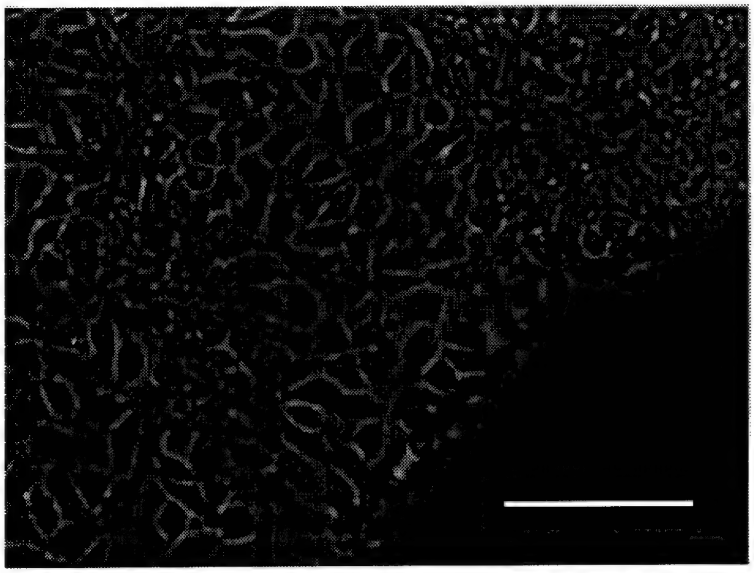

Fig. 1. Explant cultures of BRF-96T. The black area is the tissue attached to the FNC coated dish.

This picture also shows outgrowth of epithelial cells.

Photographed *without* PhaseContrast.

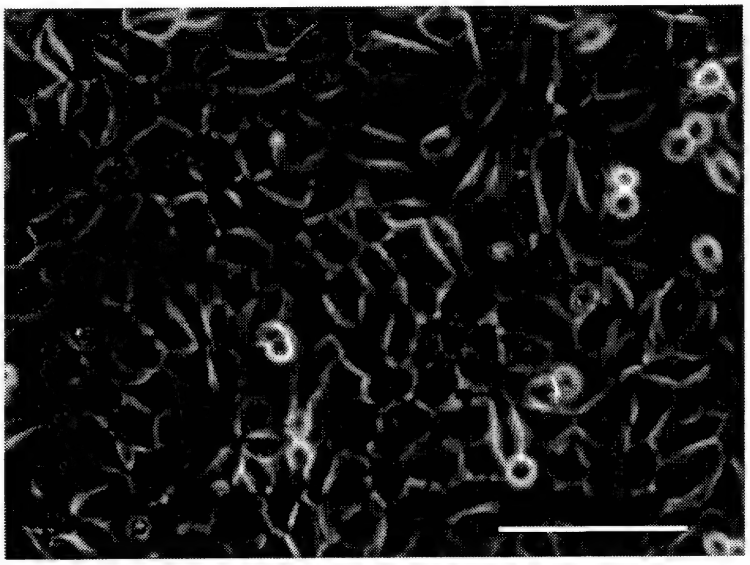

Fig. 2. Cellular outgrowth from the above explant showing dividing cells.

Photographed with Phase Contrast.

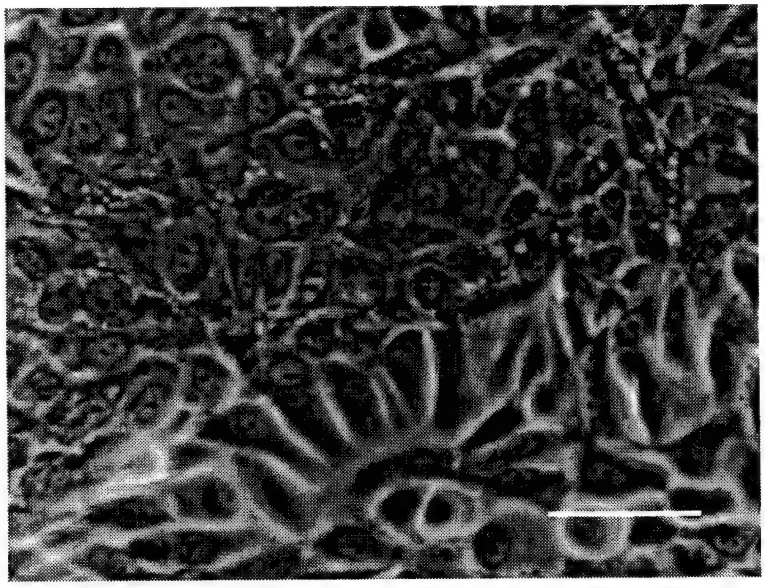

Fig. 3. Cellular outgrowth of BRF-35TA showing two cell populations.

Photographed with Phase Contrast.

The scale bar represents 0.1 mm.

LEGENDS

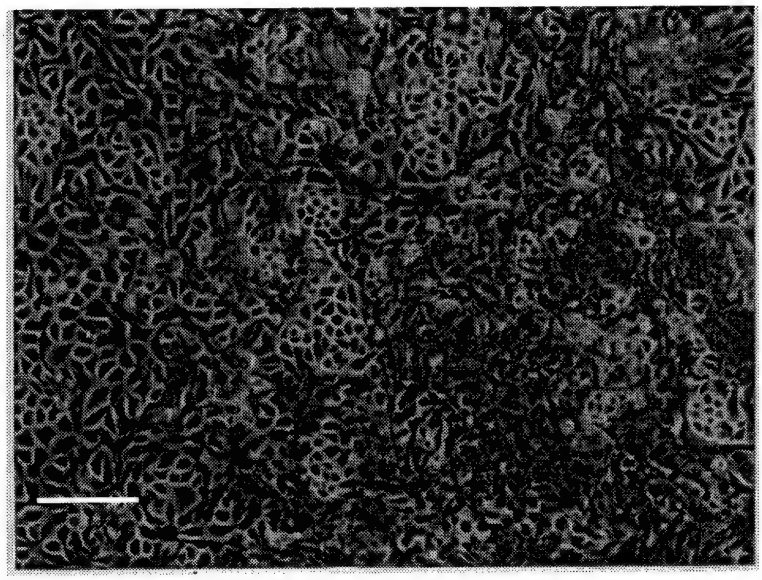

Fig. 4. Spill cell cultures of BRF-96. This picture is shows two cell types. Photographed with Phase Contrast.

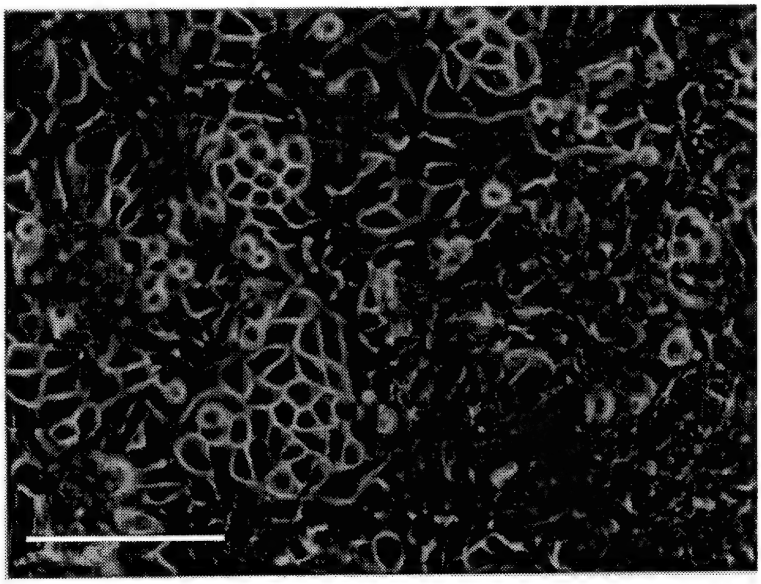

Fig. 5. Same picture as above photographed at a higher magnification using Phase Contrast.

The second cell population is reminiscent of "domes" seen in an established breast cancer cell line, MCF-7

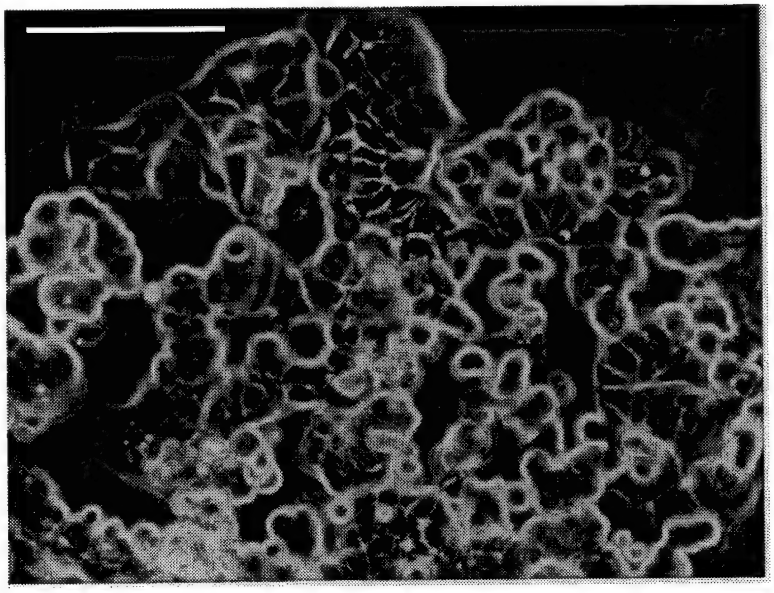

Fig. 6. Phase Contrast picture of MCF-7 cell line.

Photographed at the same magnification for comparison with Fig. 5.

The scale bar represents 0.1 mm.

LEGENDS

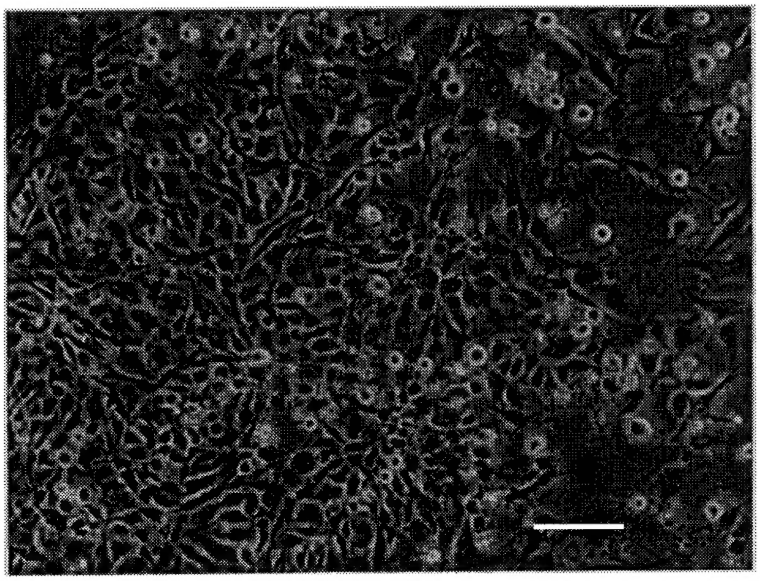

Fig. 8. BRF-71 TC at SC 14.

After initial logarithmic growth, the cells now show patchy growth.

Photographed with Phase Contrast.

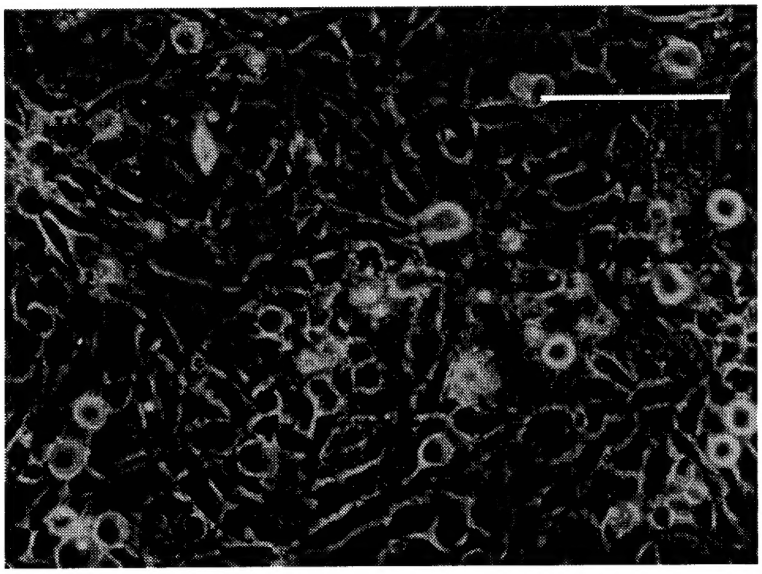

Fig. 9. Same picture as above photographed at a higher magnification using Phase Contrast.

Dividing cells as well as apoptotic cells are present in this culture.

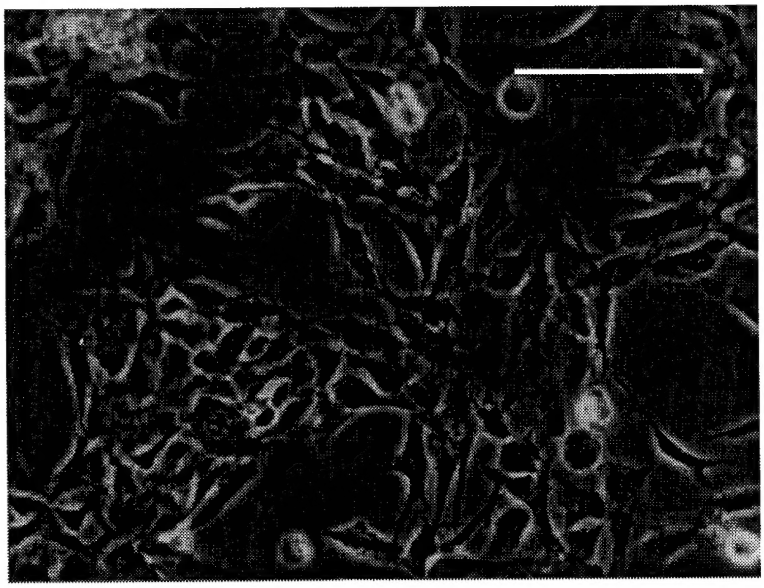

Fig. 9. BRF-71 TC at SC 5.

Phase Contrast picture of an earlier subculture reconstituted from cryopreserved stock

The scale bar represents 0.1 mm.

Chromosome Distribution in BRF 69TC SC 4

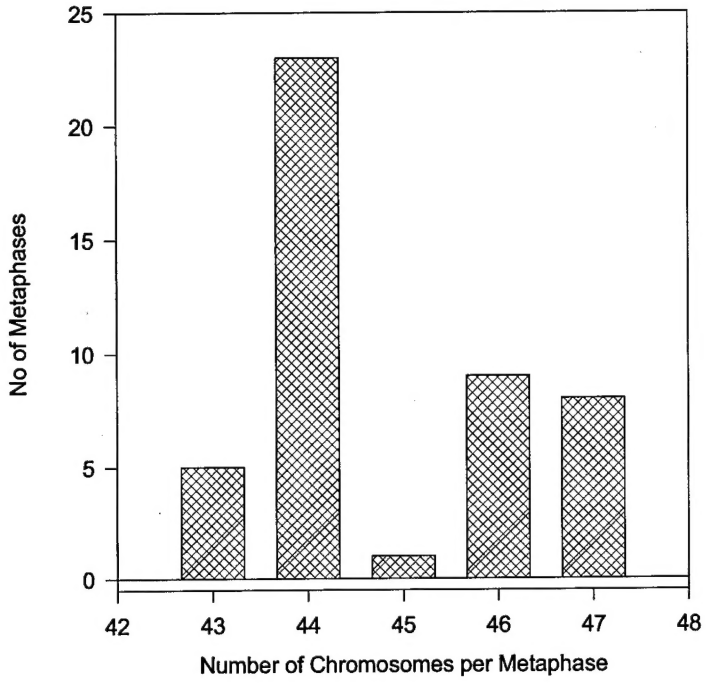

Chart 1A

One of the Giemsa banded Karyotypes from BRF 69TC (SC4)

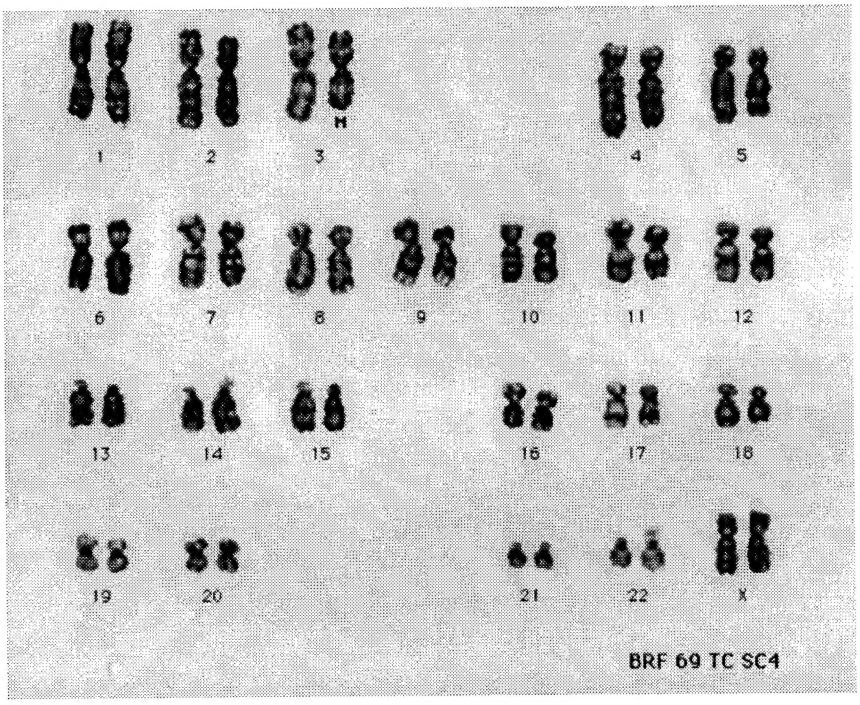

Chart 1B

Chromosome Distribution in BRF 71TC (SC5)

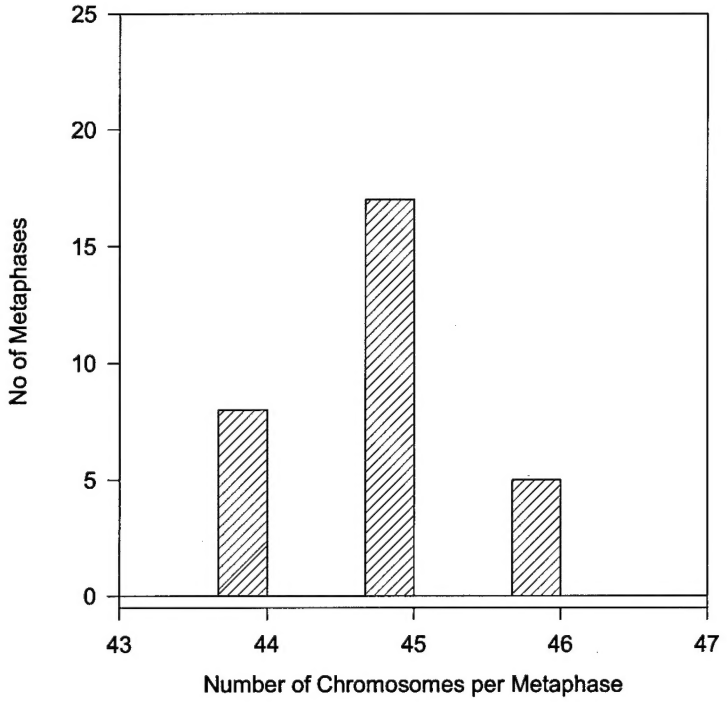

Chart 2A

One of the Giemsa banded Karyotypes from BRF 71TC (SC5)

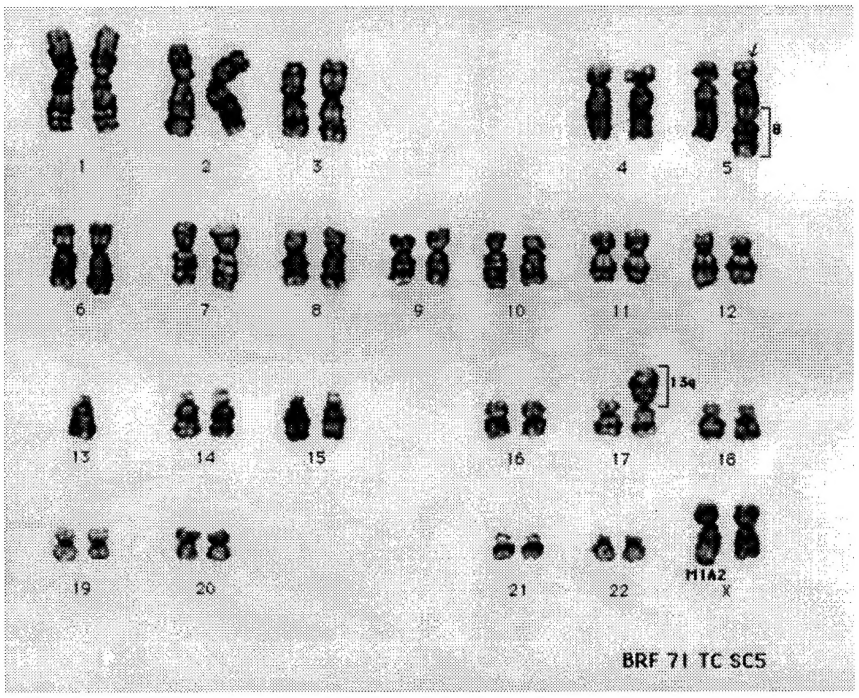

Chart 2B

Chart 3. Cumulative Population Doubling Levels of three human breast cancer cell lines.